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Final Report

Regional Measurement of Body Nitrogen

February 1, 1976 to October 31, 1976

NAS 9-14248

to
National Aeronautics and Space Administration
Lyndon B. Johnson Space Center
Houston, Texas

by
H. E. Palmer

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REGIONAL MEASUREMENT OF BODY NITROGEN

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ABSTRACT

Studies of methods for determining changes in the muscle mass of arms and legs are described. ^{13}N measurements were made in phantom and cadaver parts after neutron irradiation. The reproducibility in these measurements was found to be excellent and the radiation dose required to provide sufficient activation was determined. Potassium-40 measurements were made on persons who lost muscle mass due to leg injuries. It appears that ^{40}K measurements may provide the most accurate and convenient method for determining muscle mass changes.

INTRODUCTION

This report describes further progress in the development of methods for determining changes in muscle mass which may occur in astronauts and animals during space flight. Both the potassium and nitrogen content of the body are related to the lean tissue or muscle mass of the body and this work was limited to studying and developing methods for measuring these two elements in regional parts of the body rather than in the total body.

Of the several possible neutron activation methods for nitrogen determination, the studies described in a previous report⁽¹⁾ indicated that the ^{13}N measurement after low level neutron irradiation should be the most accurate. This method has now been studied in more detail and its usefulness is described.

In some of our earlier considerations of ^{40}K determination as a measure of muscle mass it was thought that the measurement of ^{40}K in single body parts such as the upper or lower arm or leg would be subject to poor counting precision due to low count rate and therefore be of limited usefulness. As conceptual designs of very efficient detectors were developed to reduce the required neutron radiation dose to the body part for the ^{13}N method, it became obvious that these same detectors could probably be used for ^{40}K measurement. Further testing indicated that the count rate from ^{40}K would be sufficient for precise measurements to be made so a good portion of this work was devoted to ^{40}K studies in the legs and arms of human subjects. It now appears that the ^{40}K method is more accurate and easier than the ^{13}N method and does not require any neutron irradiation.

PART I

THE USE OF ^{13}N AS A MEASURE OF MUSCLE MASS IN THE BODY AFTER NEUTRON IRRADIATION

Preparation of Phantom Body Parts

The upper and lower legs and upper and lower arms of an anthropomorphic (Remab) phantom containing human skeletal parts were filled with a solution of gelatine containing the same nitrogen content as lean beef tissue (one part gelatine powder to four parts water by weight). The gelatine solution became firm upon cooling and remained stable for several months. The gelatine is used rather than a water solution of a nitrogen salt so that the activated nitrogen remains in place as it does in the muscle of the human body. Figure 1 is a photograph of the phantom parts used.

The phantom parts are not truly representative of the tissue in human limbs since the arms and legs contain some fat and fluids which were not present in the lean beef tissue used as a standard for the gelatine preparation. Therefore, the nitrogen content of the phantom is expected to be somewhat higher than that of overall limb tissue.

Neutron Irradiation

The phantom parts were irradiated with a collimated beam of neutrons from a Kaman 711 neutron generator. The collimation restricts the irradiation and activation to only those parts of the body of specific interest. This eliminates unnecessary radiation dose to other parts of the body and counting interference from ^{13}N which would otherwise be produced in these other body sections.

A diagram of the irradiation facility is shown in Figure 2. The 14 MeV neutrons interact with the iron collimator and are either absorbed or reduced to an energy below the threshold of the $(n, 2n)$ reaction. Tissue which is behind the iron collimator is not significantly irradiated or activated. Vials of $\text{Ca}(\text{NO}_3)_2$ solution placed behind the collimator gave a ^{13}N count which was only two percent of that of a similar vial placed at the same distance away from the source but in the direct beam.

The length of irradiation is one minute. Since only relative changes in muscle mass are being measured, bilateral irradiations are not necessary if the same irradiation and counting geometrics are used for each successive measurement. The irradiation is standardized by simultaneously irradiating a standard $\text{Fe}(\text{NO}_3)_3$ solution and then measuring the amount of ^{56}Mn produced by the reaction $^{56}\text{Fe}(\text{n}, \text{p})^{56}\text{Mn}$.

^{13}N Counting

In these studies, the ^{13}N in the body part is measured by placement between two 4" thick, 9-3/8" diameter $\text{NaI}(\text{Tl})$ detectors as shown in Figure 3. Since ^{13}N decays by positron emission, two 0.51 MeV photons are simultaneously emitted in exactly opposite directions and these can be counted either directly or by the coincidence method.

The four phantom parts were irradiated and then measured in the two counting modes to determine the radiation dose necessary to produce a net count of 10,000 in twenty minutes. A 10-inch long section of the body part was irradiated for one minute, and after a twenty-minute time interval to allow decay of interfering ^{30}P , the irradiated section was centered between the two detectors and measured for twenty minutes. The distance between the detectors was varied according to the thickness of the limb. The results are shown in Table 1 and indicate that a dose of about 100 millirad (= 1 rem) would be necessary for measuring the nitrogen content in the lower arm with a counting error of about two percent at the ninety-five percent confidence level. Other sections of the limbs would require less radiation dose.

TABLE 1

^{13}N COUNTING RATE AND NECESSARY RADIATION DOSE FOR NITROGEN MEASUREMENT
USING TWO 4" X 9-3/8" DETECTORS

Body Part	Net Counts per 20 Minutes		Dose (millirads) Required to Obtain 10,000 Net Counts in 20 Minutes	
	Direct Counting	Coincidence Counting	Direct Counting	Coincidence Counting
Upper Leg	133,800	39,205	38	127
Lower Leg	76,350	38,500	65	130
Upper Arm	62,250	41,800	77	120
Lower Arm	50,850	37,700	98	133

Although this dose level is acceptable and is currently being used in in vivo neutron activation analysis in routine clinical practice in several institutions throughout the world, it should be reduced to as low a level as is practical. This can be done by increasing the counting efficiency of the detector system. An annulus shaped detector having an inside diameter of 7", a length of 10", and a NaI(Tl) thickness of 4" would significantly increase the counting efficiency. Such a detector was not available for this study and would cost about \$30,000. An estimate was made of the expected count rate from each limb in such a detector. The estimate was made by counting the thigh of a person between the two 4" x 9-3/8" detectors and then estimating the amount of natural ^{40}K present. The same amount of potassium was placed in an annulus shaped detector which had only a 3" diameter hole. The ratio of the count rate of the potassium in the leg on the two detectors to that obtained in the annulus detector from the same amount of potassium provided an estimate of ^{13}N count rate of the various limbs after neutron irradiation in a larger annulus detector. These estimates are presented in Table 2.

TABLE 2

^{13}N COUNTING RATE AND NECESSARY RADIATION DOSE FOR NITROGEN MEASUREMENT
USING AN ANNULUS DETECTOR

Body Part	Net Counts per 20 Minutes		Dose (millirads) Required to Obtain 10,000 Net Counts in 20 Minutes	
	Direct	Coincidence	Direct	Coincidence
	Counting	Counting	Counting	Counting
Upper Leg	300,000	300,000	14	14
Lower Leg	174,000	165,000	23	25
Upper Arm	144,000	180,000	28	23
Lower Arm	120,000	160,000	34	24

The data in Table 2 were calculated assuming that the annulus detector had two isolated halves so that coincidence counting could be done if desired. The use of the annular shaped detector would reduce the required dose to about one-third that needed when using two flat detectors shown in Figure 3. A dose of 25 millirad (250 millirem) should be acceptable for use in studies on space flight personnel.

Reproducibility of Limb Position in Irradiation and Counting

For this ^{13}N method to be successful the conditions for irradiation and counting must be exactly the same during each determination. The most difficult condition to reproduce is that of the position of the body part. A slight change in the orientation or lateral movement of the limb with respect to the neutron source or the detector will produce errors in the measurement. Phantom and cadaver legs are quite easily repositioned each time since they are firm and rigid and there is no muscular tension or strain. Special positioning devices are necessary to exactly reposition a limb of a living person. We have found the use of individually prepared limb molds are adequate for this purpose. A plaster of paris mold can be easily made for the full or partial length of an arm or leg. From the plaster of paris mold, thinner and less dense molds can be formed from materials such as fiberglass-reinforced epoxy or expanded rigid plastic foams. The position of the detector as well as the neutron source can be depressed into the mold so that exact geometrics and positions can be reproduced over long intervals of time. In the case of

muscle mass change where the limb may not fit the original mold because of the change in size, the positioning points of contact should emphasize bony regions such as ankles, heels, knees, elbows, and wrists where size change will be minimal.

A study was conducted to determine the effect of position change in counting ^{13}N in the upper leg section of the phantom. In moving the leg section in the direction of the knee the count rate decreased two percent for a 1/2" move and three percent for a 1" move. When moved to positions of 1/2" and 1" towards the pelvis, the count rate increased 3.5 percent and five percent, respectively. These results indicate the necessity of exactly reproducing the counting position as well as the irradiation position.

The upper leg section was irradiated and counted three times to determine the overall reproducibility. A standard $\text{Fe}(\text{NO}_3)_3$ solution was simultaneously irradiated with each run. The results shown in Table 3 indicate that reproducible measurements are possible with this method.

TABLE 3

REPRODUCIBILITY OF IRRADIATING AND COUNTING THE UPPER PHANTOM LEG

<u>Run Number</u>	<u>Net Leg Count</u>	<u>Standard Count</u>	<u>Ratio</u> $\frac{\text{Leg Count}}{\text{Standard Count}}$
1	98,257	166,788	.589
2	98,000	165,805	.591
3	100,309	167,107	.600
Range $\pm 0.9\%$			

Measurement of ^{13}N in a Cadaver Leg

The entire leg of a cadaver was obtained from the University of Washington School of Medicine. The size of the leg was somewhat larger than that of the phantom. The upper part of the leg was irradiated and counted with exactly the same procedure used to obtain the phantom leg data in Table 1. The count rate of ^{13}N obtained from the cadaver leg was only fifty-six percent of that from the phantom leg and indicates that the

nitrogen content of the cadaver leg is only slightly more than half that of the gelatine-filled phantom leg. This can probably be explained by the fact that the gelatine in the phantom represents all lean meat with most of the fluids drained or evaporated, whereas the cadaver leg contains fat and some fluids. This means that for living persons the radiation dose required to sufficiently activate the nitrogen would be about two times the dose values listed in Tables 1 and 2 for the phantom limb.

Summary of the ^{13}N Method for Determining Body Nitrogen

The studies described in this report bring this work to the point where further progress must be done in living humans. Further studies with phantoms and cadavers would be of limited use. Since Battelle-Northwest is not in a position to conduct medical studies on patients or volunteers, the logical extension of this work is to assist the physicians and scientists of the University of Washington in the testing and application of this method for human use. This procedure has been successful in the application of several in vivo neutron activation methods which were initially developed at Battelle-Northwest. Satisfactory test subjects for the ^{13}N method would be people who have had leg injuries which have required a leg cast for six weeks, or longer. The inactivity will produce a ten to twenty percent loss of muscle mass in the leg which will be slowly replaced after the cast is removed. These types of subjects are being used in the ^{40}K measurement studies described in Part II of this report.

The ^{13}N method appears to be feasible for use in measuring regional muscle mass changes which occur during space flight. The measurement would be difficult to do during space flight and will probably be restricted to pre- and post-flight measurements. The method has a few interferences which have been described in a previous report⁽¹⁾, but adequate corrections for these can be made. The most serious interference is the production of ^{13}N from oxygen in the body. This source of ^{13}N is about fifteen percent of the total produced. Since oxygen is a rather constant elemental fraction of body tissue its interference is constant and can be subtracted except for the fact that in regional measurements such as a section of leg, the ^{13}N produced from oxygen may become mobile and move out of that section before

or during the count. Also in this regard about nine percent of the nitrogen is in the blood and the ^{13}N in the blood would move out of that section of the body being measured and be diluted by the total volume of the blood. The transfer of ^{13}N out of the body part probably reaches an equilibrium by the time the count is started twenty minutes after irradiation. This affect needs to be studied with a living person.

During the period of this work it became obvious that with the large annular detectors for measuring ^{13}N described in this report, the count rate of ^{40}K would be high enough to give accurate counts on sections of the arms and legs. Studies of ^{40}K counting in these sections are described in Part II of this report, but are not complete. However, it appears that ^{40}K counting may provide a more accurate measure of muscle mass change with less interferences and no radiation dose. These ^{40}K studies should be completed before any decision is made to proceed further with the ^{13}N studies.

PART II

DETERMINATION OF MUSCLE MASS CHANGES IN LEGS AND ARMS BY ⁴⁰K MEASUREMENTS

It has been demonstrated by several investigators that the ⁴⁰K content of the body is directly proportional to the lean body mass. This has been found to be true in humans through cadaver analysis⁽²⁾ and in animals by analysis of rats⁽¹⁾. This relationship is also supported by extensive total body potassium measurements and their correlation to body composition and age⁽³⁾. The standard deviation for whole body counting measurements is about $\pm 5\%$ which is as large, or larger, than some of the muscle mass changes expected during space flight. However, if only the muscle mass change in particular parts of the body are of interest it appears that the ⁴⁰K in some of these parts can be measured with a high degree of accuracy when done with a detector of optimum size and shape.

In studies of increasing the detector size to obtain maximum counting efficiency of activated ¹³N in arms and legs it became obvious that this same detector would also count ⁴⁰K with good accuracy. The optimum size detector would be an annulus of NaI(Tl) 10" long with a center hole diameter of 7" and a wall thickness of at least 3". A detector of this size and shape was not available but two 13-1/2" diameter x 6" thick (NaI(Tl) detectors with 4" thick pure NaI light pipes were borrowed from another department. The NaI light pipes absorb essentially all of the ⁴⁰K gamma rays from the photo-multiplier tubes and therefore allow a low background for measuring ⁴⁰K in the legs. When the upper portions of both legs of a person were placed between these two detectors, a net ⁴⁰K count from the potassium in the legs was about 12,000 counts for a fifteen-minute count over a background of 4,300 counts. This should allow a standard deviation of about $\pm 1\%$ for these measurements.

These detectors were set up in the iron room of the Battelle Whole Body Counting facility and weekly measurements were started on several people who had recently started exercising at a local health spa for the purpose of developing increased body muscle. This experiment was not completely

successful and was terminated after six weeks, but did result in the following information and conclusions:

- 1) Since the thigh muscles are already under much stress from walking and carrying the weight of the body, it is difficult to significantly increase the muscle size.
- 2) The ^{40}K content in the legs showed an increase of a few to possibly six percent; however, the count rate from one time of the day to another can vary by as much as four to five percent. If a person has two successive fifteen-minute counts while laying in the counter the second count is always less than the first. If a person does deep knee bends until legs are fatigued and then is counted immediately, the count will be five or six percent higher than a count made just before the knee bends. Even though the statistical standard deviation of the counter should be $\pm 1\%$, the observed counts significantly exceeded this variation. These results indicate that blood and fluid flow into the muscles is not constant and is significantly influencing the count. There is a gradual shift of the fluids out of the legs during two successive counts and there is a shift of blood and fluids into the legs during and immediately after exercise.
- 3) The changes seem to be larger than that predicted by changes in blood flow since only 6.3 percent of total body K is in blood and all or even half of the blood could not be in the thigh muscles at one time. This indicates that a shift of ^{40}K from other parts of the body to fatigued muscles may possibly be occurring.
- 4) The results thus far indicate that ^{40}K measurement in legs is variable and depends on the condition of the legs.

Since the arms are under less stress than the legs during normal daily activities, ^{40}K measurements were made on a subject's arm muscle over a four-week period as he started and continued arm exercises. The ^{40}K content increased about five percent with a deviation of $\pm 2\%$ which is the

deviation expected from the number of counts obtained. The precision of the arm counts appeared to be much better than that for the legs and is encouraging in the use of ^{40}K as a measure of muscle mass. It may be that in order to obtain consistent and meaningful measurements on the legs as well as the arms, the measurements must be done after a period of rest when the body is in a state of equilibrium with respect to potassium.

The addition of muscle mass to arms and legs over short periods of time appears to be limited due to non-availability of subjects fully committed to this purpose. In the latter part of the ^{40}K studies, measurements have been made on the individual legs of people whose legs have been injured and have been placed in casts for a period of six weeks, or longer. Arrangements were made with a local Orthopedic and Fracture Clinic to obtain patients for study after their legs have been removed from a cast. They indicate that some of their patients lose up to two inches in circumference in their upper leg during the period in which the leg is in a cast. This corresponds to about twenty percent loss of muscle mass which is slowly replaced after the cast is removed. This provides a significant muscle mass change which should be easy to measure and study.

This study has not been completed and will continue over into the next period of funding. Measurements were started on three volunteers within a few days after their casts were removed. The measurements were made weekly for the first few weeks and then spaced at longer intervals. Figure 4 shows the results obtained through October 15, 1976. It is obvious that muscle mass in not rapidly increased in the injured legs after the cast is removed. The muscle mass may continue to decrease since the legs are still sore and painful and some of the patients continue to use crutches for a week, or two. Several problems developed with this particular group of volunteers in that Subject #1 lost his job and had to move to Alaska just as his muscle mass was showing an increase, Subject #2 broke his leg again after the fourth week and had to be put into a cast again, and Subject #3 has not been able to really use her leg as much as she should and has recently been receiving special therapy to develop this use.

Despite these problems the study looks encouraging in that it appears that muscle mass changes in the legs and probably arms can be determined by ^{40}K measurements even with the detectors we are using, which are not of the optimum size and shape. With the beginning of the new period of funding a new and larger group of leg injury volunteers will be studied. With a detector of proper size and shape, a greater precision in the leg counts will result which should allow muscle mass changes which exceed two percent to be measured. It is recommended that these ^{40}K studies be continued because of the simplicity of the method which does not require any radiation dose as the ^{13}N method does.

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2. Forbes, G.B.; Gallup, J.; and Hursh, J.B., Estimation of Total Body Fat from Potassium-40 Content. Science 133 (1961) p 101-102.
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FIGURE 1. Arm and Leg Sections
of Remab Phantom Filled with
Gelatine Solution

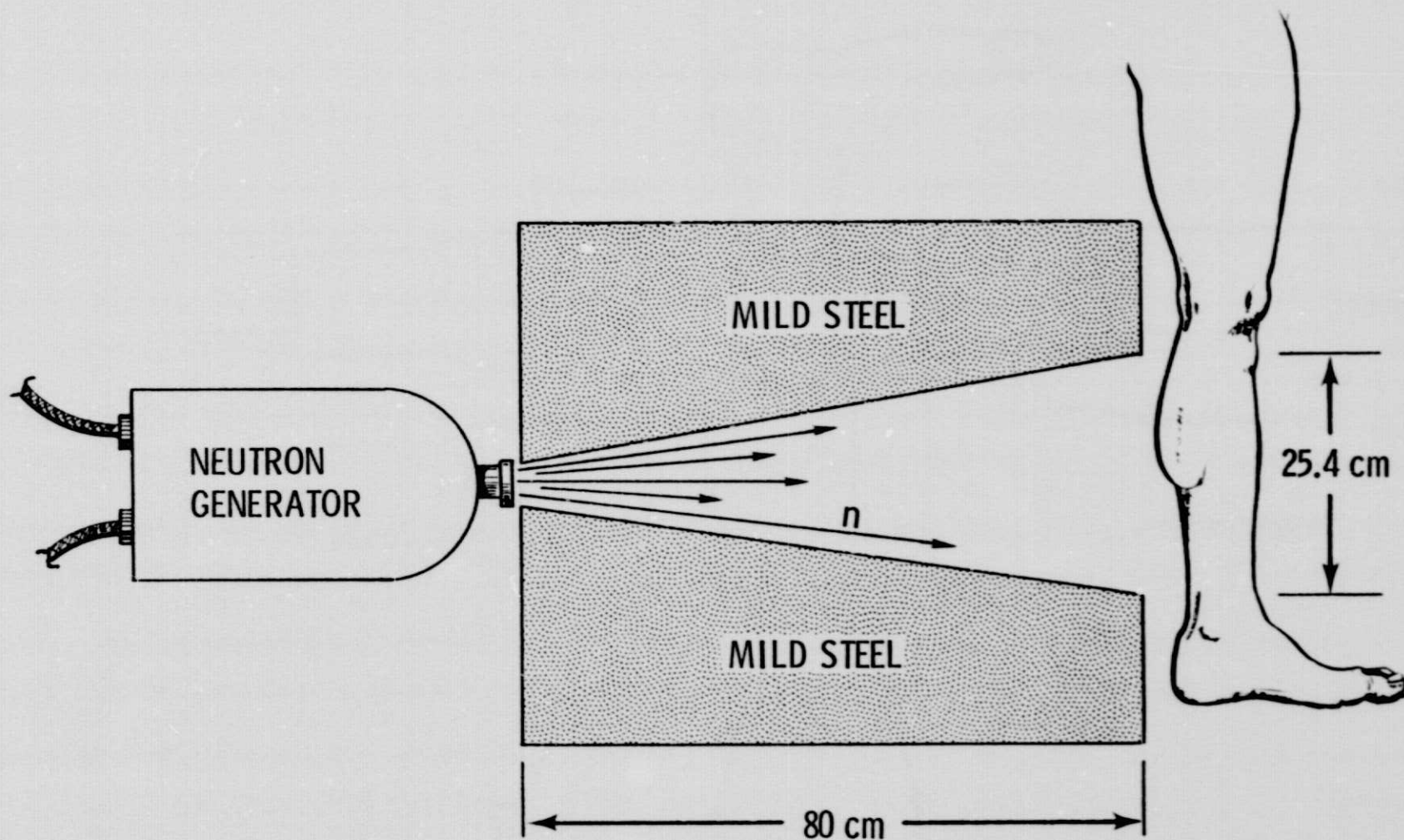


FIGURE 2. Neutron Source and Collimator Arrangement for Activating Nitrogen in Sections of the Arms and Legs

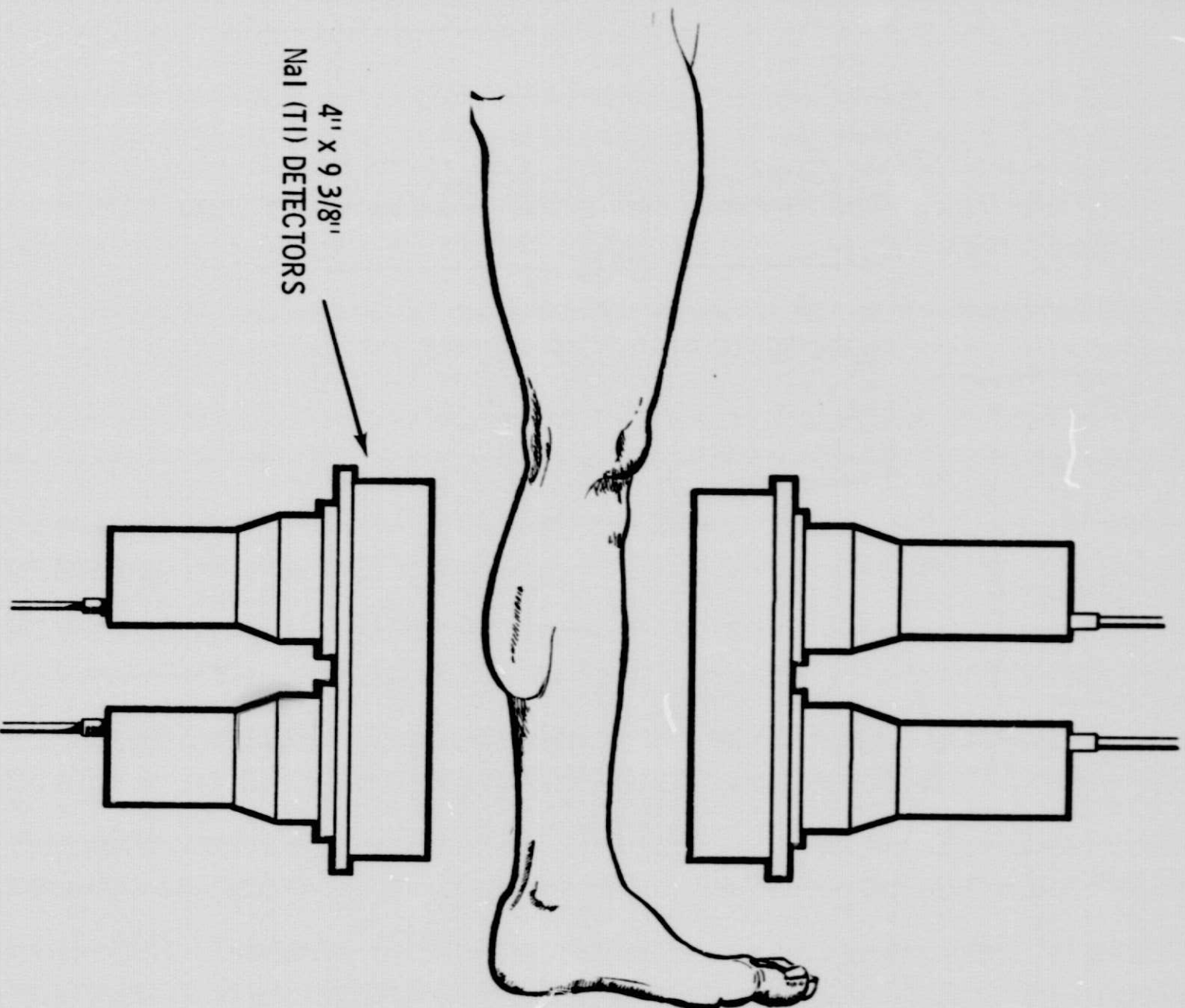


FIGURE 3. Detector Arrangement Used for Measuring ^{13}N in Sections of the Arms and Legs

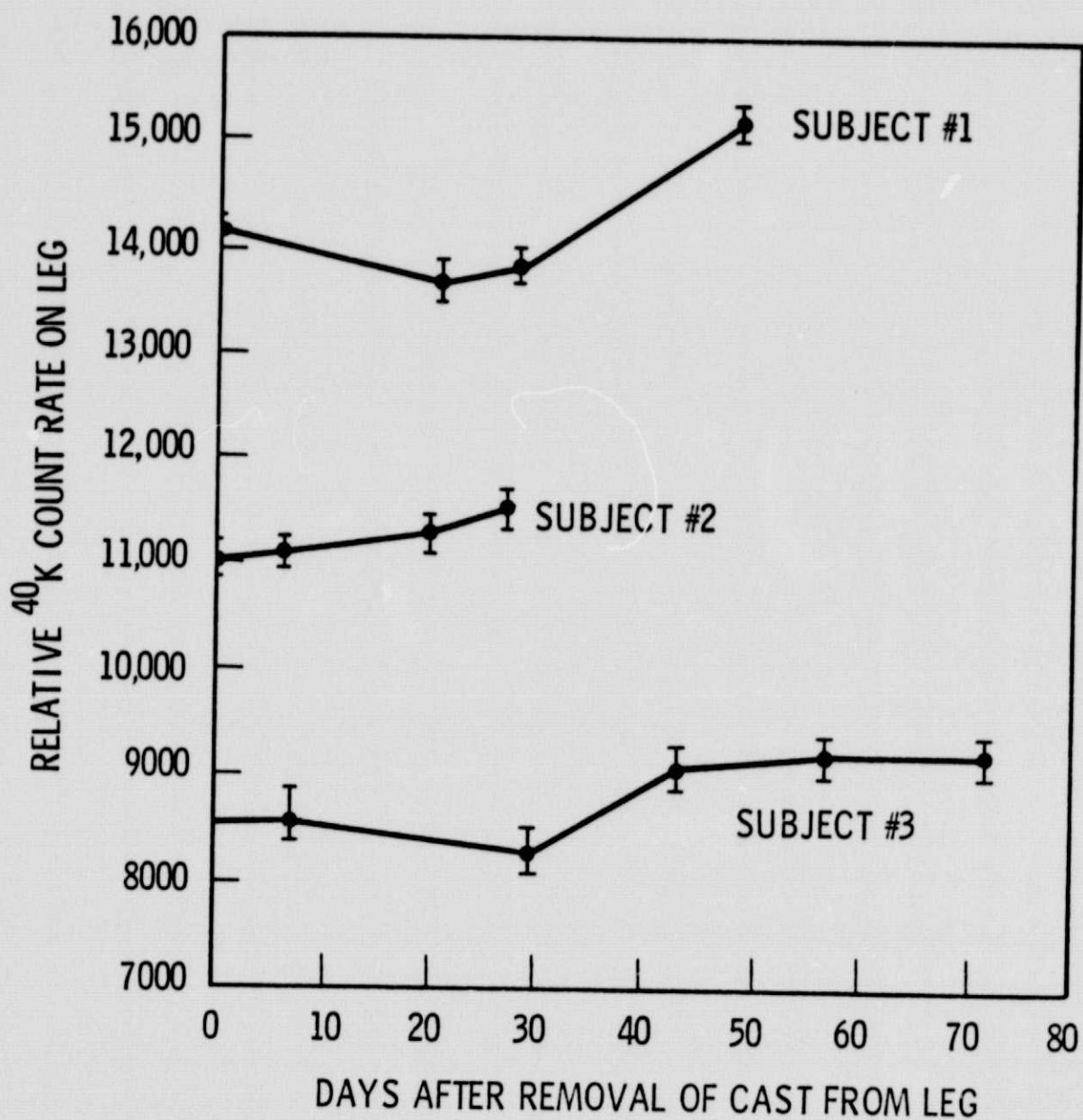


FIGURE 4. ^{40}K Content of the Thigh After Removal of Leg Cast